

AMENDMENTS

Amendments to the Specification:

At page 14, lines 13 through 20, please replace with the following paragraph:

FIG. 4. Tactics for competitive RT-PCR™ with COP primers. The portion of the HSP27 cDNA sequence (SEQ ID NO:4) indicated with the heavy underline below can be amplified by the standard primers COP 32 (SEQ ID NO:3) and COP 46 (SEQ ID NO:5). Primer CRT004, containing the COP 32 sequence, a 5 bp insert (identified by the box), and the next 8 bp from HSP27 ("clamp" sequence, identified by overline) were synthesized. When CRT004 (SEQ ID NO:6) and COP 46 were used in a PCR™ reaction containing the HSP27 template, an amplimer identified as CRT32/46 (SEQ ID NO:7) was produced. As CRT32/46 contains all of the HSP27 sequences plus the 5 bp insert it can be used as a competitive template.

At page 15, lines 14 through 21, please replace with the following paragraph:

FIG. 8. Partial sequence of MLN 62 mRNA. Primers for COP are highlighted, and the poly(A) addition signal sequence is underlined. The A-end primer sequence (CATGCCCTT), starting at position 1760, contains the CATG that is closest to the 3' end of the mRNA. The highlighted B-end primer sequence (TGAGATC), starting at position 1880, contains the first GATC following the A-end primer. Note that the actual B-end primer contains the reverse complement of the highlighted sequence (GATCTCA) (SEQ ID NO:8). This decreases the number of positions queried at the B-end by one, thus reducing the number of experiments by a factor of four.

At page 32, lines 5 through page 36, please replace Table 1 with the following Table 1:

TABLE 1: RESTRICTION ENZYMES

Enzyme Name	Recognition Sequence	SEQ ID NO:
--------------------	-----------------------------	-------------------

AatII	GACGTC	
<u>Acc65 I</u>	GGTACC	

<u>Acc</u> I	GTMKAC	
<u>Aci</u> I	CCGC	
<u>Acl</u> I	AACGTT	
<u>Afe</u> I	AGCGCT	
<u>Afl</u> II	CTTAAG	
<u>Afl</u> III	ACRYGT	
<u>Age</u> I	ACCGGT	
<u>Ahd</u> I	GACNNNNNGTC	<u>9</u>
<u>Alu</u> I	AGCT	
<u>Alw</u> I	GGATC	
<u>AlwN</u> I	CAGNNNCTG	
<u>Apa</u> I	GGGCC	
<u>ApaL</u> I	GTGCAC	
<u>Apo</u> I	RAATTY	
<u>Asc</u> I	GGCGCGCC	
<u>Ase</u> I	ATTAAT	
<u>Ava</u> I	CYCGRG	
<u>Ava</u> II	GGWCC	
<u>Avr</u> II	CCTAGG	
<u>Bae</u> I	NACNNNNGTAPyCN	<u>10</u>
<u>BamH</u> I	GGATCC	
<u>Ban</u> I	GGYRCC	
<u>Ban</u> II	GRGCYC	
<u>Bbs</u> I	GAAGAC	
<u>Bbv</u> I	GCAGC	
<u>BbvC</u> I	CCTCAGC	
<u>Bcg</u> I	CGANNNNNNTGC	<u>11</u>
<u>BciV</u> I	GTATCC	
<u>Bcl</u> I	TGATCA	
<u>Bfa</u> I	CTAG	
<u>Bgl</u> I	GCCNNNNNGGC	<u>12</u>
<u>Bgl</u> II	AGATCT	
<u>Blp</u> I	GCTNAGC	
<u>Bmr</u> I	ACTGGG	
<u>Bpm</u> I	CTGGAG	
<u>BsaA</u> I	YACGTR	
<u>BsaB</u> I	GATNNNNATC	<u>13</u>
<u>BsaH</u> I	GRCGYC	
<u>Bsa</u> I	GGTCTC	
<u>BsaJ</u> I	CCNNGG	
<u>BsaW</u> I	WCCGGW	
<u>BseR</u> I	GAGGAG	
<u>Bsg</u> I	GTGCAG	
<u>BsiE</u> I	CGRYCG	
<u>BsiHKA</u> I	GWGCWC	
<u>BsiW</u> I	CGTACG	

<u>Bsl I</u>	CCNNNNNNNGG	<u>14</u>
<u>BsmA I</u>	GTCTC	
<u>BsmB I</u>	CGTCTC	
<u>BsmF I</u>	GGGAC	
<u>Bsm I</u>	GAATGC	
<u>BsoB I</u>	CYCGRG	
<u>Bsp1286 I</u>	GDGCHC	
<u>BspD I</u>	ATCGAT	
<u>BspE I</u>	TCCGGA	
<u>BspH I</u>	TCATGA	
<u>BspM I</u>	ACCTGC	
<u>BsrB I</u>	CCGCTC	
<u>BsrD I</u>	GCAATG	
<u>BsrF I</u>	RCCGGY	
<u>BsrG I</u>	TGTACA	
<u>Bsr I</u>	ACTGG	
<u>BssH II</u>	GCGCGC	
<u>BssK I</u>	CCNGG	
<u>Bst4C I</u>	ACNGT	
<u>BssS I</u>	CACGAG	
<u>BstAP I</u>	GCANNNNTGC	<u>15</u>
<u>BstB I</u>	TTCGAA	
<u>BstE II</u>	GGTNACC	
<u>BstF5 I</u>	GGATGNN	
<u>BstN I</u>	CCWGG	
<u>BstU I</u>	CGCG	
<u>BstX I</u>	CCANNNNNNTGG	<u>16</u>
<u>BstY I</u>	RGATCY	
<u>BstZ17 I</u>	GTATAC	
<u>Bsu36 I</u>	CCTNAGG	
<u>Btg I</u>	CCPuPyGG	
<u>Btr I</u>	CACGTG	
<u>Cac8 I</u>	GCNNGC	
<u>Cla I</u>	ATCGAT	
<u>Dde I</u>	CTNAG	
<u>Dpn I</u>	GATC	
<u>Dpn II</u>	GATC	
<u>Dra I</u>	TTTAAA	
<u>Dra III</u>	CACNNNGT	
<u>Drd I</u>	GACNNNNNGTC	<u>17</u>
<u>Eae I</u>	YGGCCR	
<u>Eag I</u>	CGGCCG	
<u>Ear I</u>	CTCTTC	
<u>Eci I</u>	GGCGGA	
<u>EcoN I</u>	CCTNNNNNAGG	<u>18</u>
<u>EcoO109 I</u>	RGGNCCY	

<u>EcoR I</u>	GAATTC	
<u>EcoR V</u>	GATATC	
<u>Fau I</u>	CCCGC _{NNNN}	
<u>Fnu4H I</u>	GCNGC	
<u>Fok I</u>	GGATG	
<u>Fse I</u>	GGCCGGCC	
<u>Fsp I</u>	TGCGCA	
<u>Hae II</u>	RGC _{GCY}	
<u>Hae III</u>	GGCC	
<u>Hga I</u>	GACGC	
<u>Hha I</u>	GC _{GC}	
<u>Hinc II</u>	GTYRAC	
<u>Hind III</u>	AAGCTT	
<u>Hinf I</u>	GANTC	
<u>HinP1 I</u>	GC _{GC}	
<u>Hpa I</u>	GT _{TAAC}	
<u>Hpa II</u>	CCGG	
<u>Hph I</u>	GGTGA	
<u>Kas I</u>	GGCGCC	
<u>Kpn I</u>	GGTACC	
<u>Mbo I</u>	GATC	
<u>Mbo II</u>	GAAGA	
<u>Mfe I</u>	CAATTG	
<u>Mlu I</u>	ACGCGT	
<u>Mly I</u>	GAGTC _{NNNN}	<u>19</u>
<u>Mnl I</u>	CCTC	
<u>Msc I</u>	TGGCCA	
<u>Mse I</u>	TTAA	
<u>Msl I</u>	CAY _{NNNNRTG}	<u>20</u>
<u>MspA1 I</u>	CMGCKG	
<u>Msp I</u>	CCGG	
<u>Mwo I</u>	GC _{NNNNNNNGC}	<u>21</u>
<u>Nae I</u>	GCCGGC	
<u>Nar I</u>	GGCGCC	
<u>Nci I</u>	CCSGG	
<u>Nco I</u>	CCATGG	
<u>Nde I</u>	CATATG	
<u>NgoMI V</u>	GCCGGC	
<u>Nhe I</u>	GCTAGC	
<u>Nla III</u>	CATG	
<u>Nla IV</u>	GGNNCC	
<u>Not I</u>	GC _{GGCCGC}	
<u>Nru I</u>	TCGCGA	
<u>Nsi I</u>	ATGCAT	
<u>Nsp I</u>	RCATGY	
<u>Pac I</u>	TTAATTAA	

<u>PaeR7 I</u>	CTCGAG	
<u>Pci I</u>	ACATGT	
<u>PflF I</u>	GACNNNGTC	
<u>PflM I</u>	CCANNNNNTGG	<u>22</u>
<u>PleI</u>	GAGTC	
<u>Pme I</u>	GTTTAAAC	
<u>Pml I</u>	CACGTG	
<u>PpuM I</u>	RGGWCCY	
<u>PshA I</u>	GACNNNNGTC	<u>23</u>
<u>Psi I</u>	TTATAA	
<u>PspG I</u>	CCWGG	
<u>PspOM I</u>	GGGCC	
<u>Pst I</u>	CTGCAG	
<u>Pvu I</u>	CGATCG	
<u>Pvu II</u>	CAGCTG	
<u>Rsa I</u>	GTAC	
<u>Rsr II</u>	CGGWCCG	
<u>Sac I</u>	GAGCTC	
<u>Sac II</u>	CCCGGG	
<u>Sal I</u>	GTCGAC	
<u>Sap I</u>	GCTCTTC	
<u>Sau3A I</u>	GATC	
<u>Sau96 I</u>	GGNCC	
<u>Sbf I</u>	CCTGCAGG	
<u>Sca I</u>	AGTACT	
<u>ScrF I</u>	CCNGG	
<u>SexA I</u>	ACCWG GT	
<u>SfaN I</u>	GCATC	
<u>Sfc I</u>	CTRYAG	
<u>Sfi I</u>	GGCCNNNNNGGCC	<u>24</u>
<u>Sfo I</u>	GGCGCC	
<u>SgrA I</u>	CRCCGGY G	
<u>Sma I</u>	CCCGGG	
<u>Sml I</u>	CTYRAG	
<u>SnaB I</u>	TACGTA	
<u>Spe I</u>	ACTAGT	
<u>Sph I</u>	GCATGC	
<u>Ssp I</u>	AATATT	
<u>Stu I</u>	AGGCCT	
<u>Sty I</u>	CCWWGG	
<u>Swa I</u>	ATTTAAAT	
<u>Taq I</u>	TCGA	
<u>Tfi I</u>	GAWTC	
<u>Tli I</u>	CTCGAG	
<u>Tse I</u>	GCWGC	
<u>Tsp45 I</u>	GTSAC	

<u>Tsp509 I</u>	AATT	
<u>TspR I</u>	CAGTG	
<u>Tth111 I</u>	GACNNNGTC	
<u>Xba I</u>	TCTAGA	
<u>Xcm I</u>	CCANNNNNNNTGG	<u>25</u>
<u>Xho I</u>	CTCGAG	
<u>Xma I</u>	CCCGGG	
<u>Xmn I</u>	GAANNNNTTC	<u>26</u>

At page 53, lines 5 through 20, please replace with the following paragraph:

2. Specificity Of COP

As an example of the specificity of the method, reactions were performed with primers predicted to produce a 291 bp amplimer from the murine *Brcal* gene. As template for these reactions, mRNA was prepared from cultures of mouse keratinocytes. Epidermal keratinocyte cultures were derived from newborn mice and maintained as described, Pierce *et al.*, 1998a. Total RNA was prepared by extraction into a chaotropic salt solution and organic solvent extraction using either a QIAGEN (Valencia, CA). mRNA was prepared using a QIAGEN kit, and double-stranded cDNA was synthesized using a GIBCO/BRL kit but substituting biotinylated p(dT)₁₈ as the primer for first strand synthesis. Double stranded linkers with overhangs complementary to the ends created by restriction with Nla III (A-linker) and Dpn II (B-linker) were prepared separately by mixing equal amounts of the following oligonucleotides, warming to 90°C for 2 min and slowly cooling to room temperature: A-linker - 5'-CGTCTAGACAGC (SEQ ID NO:27) (previously phosphorylated with T4 polynucleotide kinase) and 5'- GCTGTCTAGACGCATG (SEQ ID NO:28); B-linker - 5' - CGGTGATGCATC (SEQ ID NO:29) and 5' -GATCGATGCATCACCG (SEQ ID NO:30) (previously phosphorylated with T4 polynucleotide kinase).

At page 54, lines 3 through 11, please replace with the following paragraph:

To test the selectivity of the method, two pairs of primers that differed by a single nucleotide from the *Brcal* primers were also chosen that were expected to produce amplimers of 117 and 197 bp from the genes for annexin III and an anonymous cDNA (clone 2C11B), respectively. Two sets of primers for COP PCR™ reactions were synthesized, corresponding to

the A- and B- linkers above, but containing 3 or 4 nucleotide specificity regions at the 3' end. The sequences of these primer sets were:

A-end (256 primers)-5'-GCTGTCTAGACGCATGNNNN (SEQ ID NO:31);

B-end (64 primers)-5'-CGGTGATGCATCGATCNNN (SEQ ID NO:32).

At page 69, lines 1 through 18, please replace with the following paragraph:

was adsorbed to magnetic beads coated with streptavidin (Dynal, Lake Success, NY) and non-biotinylated fragments were washed from the beads. cDNA fragments still bound to the beads were ligated to the B-linker; then digested with Nla III; and fragments released from the beads by this treatment were selected and ligated to the A-linker. Double stranded linkers with overhangs complementary to the ends created by restriction with Nla III (A-linker) and Dpn II (B-linker) were prepared separately by mixing equal amounts of the following oligonucleotides: warming to 90°C for 2 min and slowly cooling to room temperature: A-linker - 5'-CGTCTAGACAGC (SEQ ID NO:27) (previously phosphorylated with T4 polynucleotide kinase) and 5'-GCTGTCTAGACGCATG (SEQ ID NO:28); B-linker - 5' - CGGTGATGCATC (SEQ ID NO:29) and 5' - GATCGATGCATCACCG (SEQ ID NO:30) (previously phosphorylated with T4 polynucleotide kinase). Linkers (217 ng) were added to restricted cDNA fragments (initially 1.5 µg), warmed to 50°C for 2 min, cooled to room temperature for 15 min, then cooled on ice. Ligation was accomplished by adding 10 U T4 DNA ligase (GIBCO/BRL) and incubating in a final volume of 50 µL for 2h at 16°C. These fragments of cDNA, containing the gene-specific targets ligated to the B and A linkers, are referred to as B/A genetags. A second preparation, A/B genetags, is obtained when Nla III restriction and A-linker ligation preceded the Dpn II restriction and B-linker ligation. Also refer to FIG. 1.

Please delete the current Sequence Listing and insert therefor the Substitute Sequence Listing numbered pages 1 to 11 as submitted electronically herewith as text.